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14. ABSTRACT This study aims to take a new approach to studying this process at the level of the translating ribosome and its associated proteins (i.e. the riboproteome). While the conventional wisdom has been that ribosome composition is absolutely fixed, we have been pursuing a line of investigation showing that it is, in fact, flexible and dynamic. Moreover, it has become apparent that the deregulation of the ribosome is implicated in disease initiation and progression, and could serve as a potential target for therapeutic intervention. Our objective is to analyze the riboproteome in a high-throughput manner in order to gain a global snapshot of all proteins that constitute the riboproteome, to evaluate which of these proteins are altered between different prostate cancer cell lines and types, and to uncover how the riboproteome is altered during prostate cancer development and progression. In addition our studies aim to understand how the riboproteome responds to androgen signaling, and the use of PI3-kinase and MAP-kinase inhibitors, each of which are clinically relevant therapeutic options for prostate cancer patients.					
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1. INTRODUCTION:

In our study, we aim further develop and refine an experimental platform to study the composition of the ribosome and its associated proteins using a mass spectrometry approach to systematically analyze the riboproteome of prostate cancer cells. This methodology has already been optimized in our lab (Reschke et al., 2013), where profound differences in the riboproteomes of normal and cancer cells have been uncovered. These data suggest that the riboproteome and its associated translational landscape are altered during transformation and that significant differences exist between cancer cells. Additionally, we identified various novel proto-oncogenic regulators of translation that have a direct implication for the etiology of prostate cancer including the RNA-binding protein Musashi and the myristoylated alanine-rich C-kinase substrate (MARCKS). Through our studies outlined here we now establish MARCKS to be an important regulatory protein contributing to control of translation in prostate cancer.

2. KEYWORDS:

Prostate cancer, translation, riboproteome, TMT mass spectrometry, iTRAQ

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of this project are to establish the importance and relevance of translation and components of the riboproteome for human prostate cancer. In particular our efforts are focused on the following specific goals

Goal 1: To profile and stage prostate cancer riboproteomes using prostate cancer cell lines that represent a spectrum of early to late stage prostate cancer.

Goal 2: To define how androgen signaling and pharmacological inhibition of key signaling pathways impact the riboproteome.

Goal 3: To validate differential components of the riboproteome in primary prostate cancer specimens and patient-derived xenografts.

What was accomplished under these goals?

Major Activities:

Complete analysis of riboproteomic changes in different prostate cancer cell lines compared to normal prostate epithelial cell lines. In order to further refine our protocol for the characterization of the prostate riboproteome we prepared a “*prostate specific super mix*” that is prepared from polysome fractions isolated from the prostate cell lines, and which were used as a common standard to compare all our samples to. To generate this control we evaluated the following cell lines: PC3, Du145, LNCaP, 22Rv1, CA HPV-10, RWPE1, PWR1E, WPE1-NB14, WPE1-NB26. This set represents a diversity of currently available prostate cell lines, including prostate cancer cell lines (PC3, Du145, R22v1, LNCaP, CA HPV-10), transformed tumorigenic cell lines derived from RWPE1 cell line (NB-14 and NB-26), and normal prostate epithelial cells (RWPE1 and PWR1E). To reflect a range of prostate cancer progression and androgen sensitivity, we used both androgen-responsive prostate cancer cell lines, such as LNCaP and 22RV1, and androgen-irresponsive, such as DU145 and PC3. Further, these cell lines differ in their metastatic potential with PC3 cells having a high [metastatic](#) potential compared to [DU145](#) cells, which have a moderate metastatic potential, and to [LNCaP](#) cells, which have a low metastatic potential. The use of this combined reference polysome lysate allowed us to directly compare the riboproteome of all prostate cancer cells and correlate the relative abundance of riboproteomic

components between datasets. In addition, we identified and validated a number of proteins previously not known to be associated with actively translating ribosomes (e.g., MARCKS), showing the potential of these data sets to identify novel regulators of translation.

Complete analysis of riboproteomic changes during prostate cancer progression. In order to characterize more specifically the riboproteomic changes that occur during prostate cancer progression *in vitro* we used a well-characterized panel of tumorigenic cell lines derived from RWPE1 prostatic epithelial cells after exposure to N-methyl-N-nitrosourea (MNU) (these cell lines are commercially available from ATCC). This family of cell lines mimics multiple steps in tumor progression from normal epithelium to PIN to invasive cancer (WPE1-NA22, WPE1-NB14, WPE1-NB11, WPE1-NB26 in order of increasing malignancy) and allows for a detailed analysis of how the riboproteome changes with increasing malignancy (Webber et al., 2001). We applied a comprehensive bioinformatics analysis of the datasets obtained in collaboration with Dr. Steve Carr at the Broad Institute of MIT and Harvard to determine what kinds of riboproteome changes may be associated with increasing prostate cancer aggressiveness and tumor progression.

Define how androgen signaling impact the riboproteome. Given that prostate riboproteomes display profound changes during tumor development (Reschke et al., 2013) we hypothesize that such changes may dictate the response to androgen and be an important mechanism in the development of resistance to this therapy. We applied a comprehensive bioinformatics analysis of the datasets to stratify the data based on androgen sensitivity of each cell line. These data will have important implications for our understanding of how modulation of riboproteome composition may be utilized to develop novel therapeutic modalities for the treatment of prostate cancer.

Validation and further characterization of identified riboproteomic component MARCKS. A major activity of the last period has also been the further characterization of the role of the riboproteomic component MARCKS in translation and how it may function in the context prostate cancer. We now have strong mechanistic data that establish an important role for this protein in regulating cellular translation.

Significant Results

We performed tandem mass tag (TMT) mass spectrometry analysis of an extensive subset of prostate cancer cell lines and normal epithelial cell lines PC3, Du145, LnCaP, 22Rv1, CA HPV-10, RWPE1, PWR1E, WPE1-NB14, WPE1-NB26 in order to identify the riboproteomic changes in prostate cancer. Figure 1 outlines the experimental strategy, by which each of the 9 cell lines were labelled for simultaneous analysis by mass spectrometry. A pooled reference sample (Pooled Ref CT2) was utilized for normalization of the data. In total we were able to identify 3,164 proteins in the isolated ribosomal fractions. As expected the 81 large ribosomal subunit (Rpl family) and small ribosomal subunit (Rps family) proteins were all identified in our analysis, and (Table 1). The remaining 3,083 proteins identified represents a two-fold increase on proteins detected by this analysis over our previously published approach, whereby 1,499 proteins in total were identified in our analysis. In addition, these data represent a much more comprehensive dataset incorporating almost all available prostate cancer cell lines.

Table 1. Identified Proteins and Ribosomal proteins.

	TMT10	TMT10	Total	Overlap in between
	Exp 1	Exp 2	(sum of 2 exp)	2 experiments
Proteins (all)	3526	3463	3731	3262
Proteins (≥ 2 peptides)	2868	2792	3462	3164
Ribosomal Proteins (≥ 2 peptides)	81	81	81	81

Experimental Strategy: 2 x TMT 10-plex experiments with 9 cell lines and pooled reference control in each of the experiment

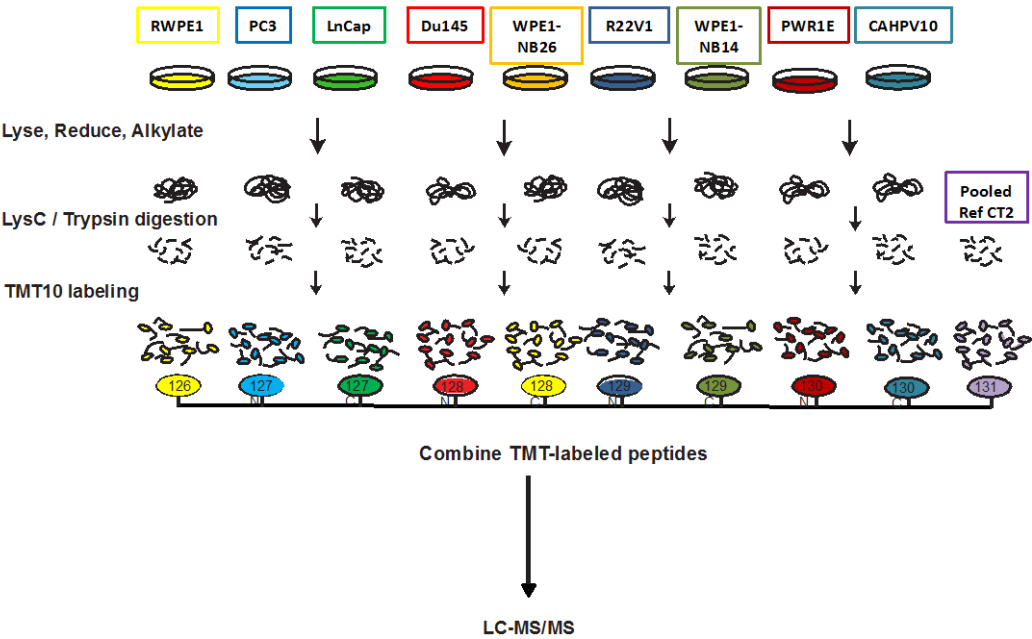


Figure 1. Outline of the experimental strategy for TMT mass spectrometry.

Furthermore, the data analysis of two replicates shows a significant enrichment in ribosomal proteins confirming a quality of sample preparation of ribosomal fractions (Fig. 2). Additionally, our data set is highly enriched in factors that relate directly to the ribosome, translational initiation and elongation validating the approach to identify factors associated with active translation and allowing us to directly compare the global translational machinery between each of these cell lines.

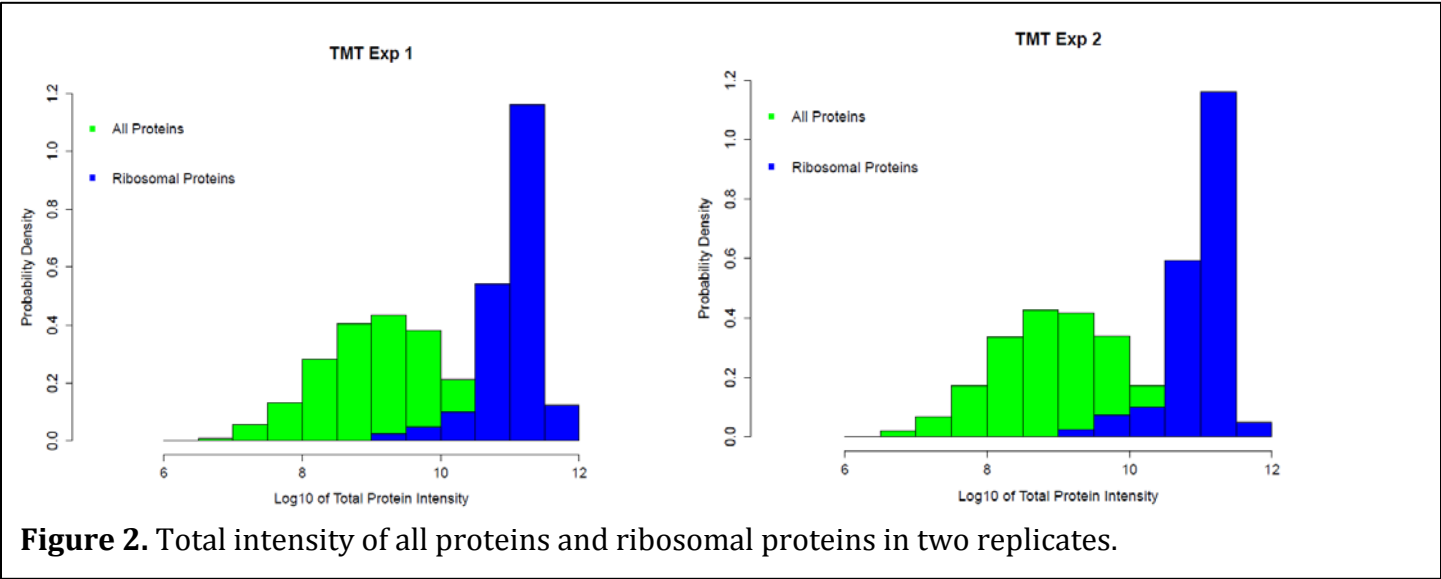


Figure 2. Total intensity of all proteins and ribosomal proteins in two replicates.

Computational analysis of our datasets showed that virtually all detected 40S and 60S ribosomal proteins and translation-associated proteins are up-regulated to some extent (up to 2-fold increase) in prostate cancer cell lines (PC3, Du145, R22v1, LnCaP) and MNU-treated WPE1 cell lines (NB-14 and NB-26) relative to normal prostate epithelial cells (RWPE1 and PWR1E) (Table 2 and Fig. 3). Interestingly, the prostate cancer cell line CA-HPV-10 appears to be an outlier and clusters with the more normal immortalized prostate epithelial cells (RWPE1 and PWR1E) (Fig. 3). These data maybe in line with the fact that the CA-HPV-10 cell line was derived from cells taken from a prostatic adenocarcinoma of Gleason Grade 4/4 and subsequently transformed by transfection with human papillomavirus 18 (HPV-18).

MNU-treated WPE1 cell lines (NB-14 and NB-26) also show some upregulation of ribosomal proteins and translation associated proteins when compared to the immortalized prostate epithelial cell line RWPE1 from which they were derived, but not to the extent observed in the true cancer cell lines PC3, Du145, R22v1, and LnCaP, and therefore cluster together between normal and cancer cell lines (Fig. 3).

On the other hand, mitochondrial ribosomal proteins are down-regulated in prostate cancer cell lines (PC3, Du145, R22v1, LnCaP) and MNU-treated WPE1 cell lines (NB-14 and NB-26) relative to normal prostate epithelial cells (RWPE1 and PWR1E) (Fig. 4).

Table 2. Ribosome associated proteins analysis in prostate cancer cell lines (PC3, Du145, R22v1, LnCaP) and MNU-treated WPE1 cell lines (NB-14 and NB-26) relative to normal prostate epithelial cells (RWPE1 and PWR1E).

Upregulated ribosomal proteins	Upregulated translation-associated proteins	Down-regulated mitochondrial ribosomal
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				proteins
RPS29	40S ribosomal protein S29	AARS	Alanine--tRNA ligase, cytoplasmic	MRPL28 39S ribosomal protein L28, mitochondrial
RPLP0	60S acidic ribosomal protein P0	EEF1B2	Elongation factor 1-beta	MRPL32 39S ribosomal protein L32, mitochondrial
RPL5	60S ribosomal protein L5	EEF1A2	Elongation factor 1-alpha 2	MRPL42 39S ribosomal protein L42, mitochondrial
RPS3A	40S ribosomal protein S3a	NARS	Asparagine--tRNA ligase, cytoplasmic	MRPS33 28S ribosomal protein S33, mitochondrial
RPL10A	60S ribosomal protein L10a	EEF2	Elongation factor 2	MRPL1 39S ribosomal protein L1, mitochondrial
RPL10	60S ribosomal protein L10	TARS	Threonine--tRNA ligase, cytoplasmic	MRPL41 39S ribosomal protein L41, mitochondrial
RPL8	60S ribosomal protein L8	TMA7	Translation machinery-associated protein 7	MRPS10 28S ribosomal protein S10, mitochondrial
RPS4X	40S ribosomal protein S4, X isoform	YARS	Tyrosine--tRNA ligase, cytoplasmic	MRPL14 39S ribosomal protein L14, mitochondrial
RPL35A	60S ribosomal protein L35a	RBMS1	RNA-binding motif, single-stranded-interacting protein 1	MRPL34 Ribosomal protein L34
RPL35	60S ribosomal protein L35	EEF1G	Elongation factor 1-gamma	MRPL24 39S ribosomal protein L24, mitochondrial
RPL32	60S ribosomal protein L32			MRPS28 28S ribosomal protein S28, mitochondrial
RPL17	Isoform 3 of 60S ribosomal protein L17	EIF5A	Eukaryotic translation initiation factor 5A-1	MRPL33 39S ribosomal protein L33, mitochondrial
RPL28	60S ribosomal protein L28	SARS	Serine--tRNA ligase, cytoplasmic	
RPL15	60S ribosomal protein L15	EEF1A1P5	Putative elongation factor 1-alpha-like 3	
RPL36A	60S ribosomal protein L36a	EIF2B1	Translation initiation factor eIF-2B subunit alpha	

RPL36	60S ribosomal protein L36	WARS	Tryptophan--tRNA ligase, cytoplasmic
RPL30	60S ribosomal protein L30	EIF4A1	Eukaryotic initiation factor 4A-I
RPL3	60S ribosomal protein L3	LSM1	U6 snRNA-associated Sm-like protein LSM1
GNB2L1	Guanine nucleotide-binding protein subunit beta-2-like 1	EIF4G1	Isoform 8 of Eukaryotic translation initiation factor 4 gamma 1
UBA52	Ubiquitin-60S ribosomal protein L40	RBM14	RNA-binding protein 14
RPS15A	40S ribosomal protein S15a	ICE2	Little elongation complex subunit 2
RPS28	40S ribosomal protein S28	EIF4B	Eukaryotic translation initiation factor 4B
RPS26	40S ribosomal protein S26	EIF1	Eukaryotic translation initiation factor 1
RPS3	40S ribosomal protein S3	EIF4E	Isoform 2 of Eukaryotic translation initiation factor 4E
RPL11	60S ribosomal protein L11	STAU2	Double-stranded RNA-binding protein Staufen homolog 2
RPL23	60S ribosomal protein L23	EIF4H	Eukaryotic translation initiation factor 4H
RPS13	40S ribosomal protein S13	EIF5	Eukaryotic translation initiation factor 5
RPL7A	60S ribosomal protein L7a	PURA	Transcriptional activator protein Pur-alpha
RPL34	60S ribosomal protein L34	EIF5B	Eukaryotic translation initiation factor 5B
RPS18	40S ribosomal protein S18	PURB	Transcriptional activator protein Pur-beta
RPS15	40S ribosomal protein S15	RBM14	Isoform 5 of RNA-binding protein 14
RPL7	60S ribosomal protein L7	SBDS	Ribosome maturation protein SBDS
RPSA	40S ribosomal protein SA	EIF3I	Eukaryotic translation initiation factor 3 subunit I
RPS25	40S ribosomal protein S25	EIF2A	Eukaryotic translation

		initiation factor 2A	
RPS21	40S ribosomal protein S21		
RPS19	40S ribosomal protein S19		
RPS16	40S ribosomal protein S16		
RPS27	40S ribosomal protein S27		
RPS11	40S ribosomal protein S11		
RPS7	40S ribosomal protein S7		
RPS5	40S ribosomal protein S5		
RPS2	40S ribosomal protein S2		
RPL27A	60S ribosomal protein L27a		
RPS9	40S ribosomal protein S9		
RPS12	40S ribosomal protein S12		
RPL14	60S ribosomal protein L14		
RPL29	60S ribosomal protein L29		
RPL4	60S ribosomal protein L4		
RPL23A	60S ribosomal protein L23a		
RPL37A	60S ribosomal protein L37a		
RPL31	60S ribosomal protein L31		
RPL13A	60S ribosomal protein L13a		
RPL6	60S ribosomal protein L6		
RPS14	40S ribosomal protein S14		
RPL9	60S ribosomal protein L9		
RPS10	40S ribosomal protein S10		
RPL26	60S ribosomal protein L26		
RPL18	60S ribosomal protein L18		
RPL38	60S ribosomal protein L38		
RPLP1	60S acidic ribosomal protein P1		

RPL21	60S ribosomal protein L21	
RPL27	60S ribosomal protein L27	
RPL18A	60S ribosomal protein L18a	
RPL24	60S ribosomal protein L24	
RPL10	60S ribosomal protein L10	
RPL37	60S ribosomal protein L37	

Some of the pro-oncogenic proteins, including several isoforms of dual specificity mitogen-activated protein kinase (MAPKs), cyclin-dependent kinase 1 (CDK1), and dual specificity protein phosphatase 3 (DUSP3) are clearly up-regulated in prostate cancer cell lines (PC3, Du145, R22v1, LnCaP) relative to normal prostate epithelial cells (RWPE1 and PWR1E) (Fig. 5 and Fig. 6). Interestingly, Arnoldussen et al (Cancer Res. 2008, 68(22)) showed that DUSP3 is up-regulated by androgens during inhibition of apoptosis in LNCaP cells, suggesting that DUSP3 has a direct role in the inhibition of JNK-dependent apoptosis in LNCaP cells and may therefore have a role in prostate cancer progression. CDK1 overexpression has been noted to correlate with several human cancers, including breast and ovarian cancer (Johnson et al, *Nature Medicine* **17**, 875–882 (2011)).

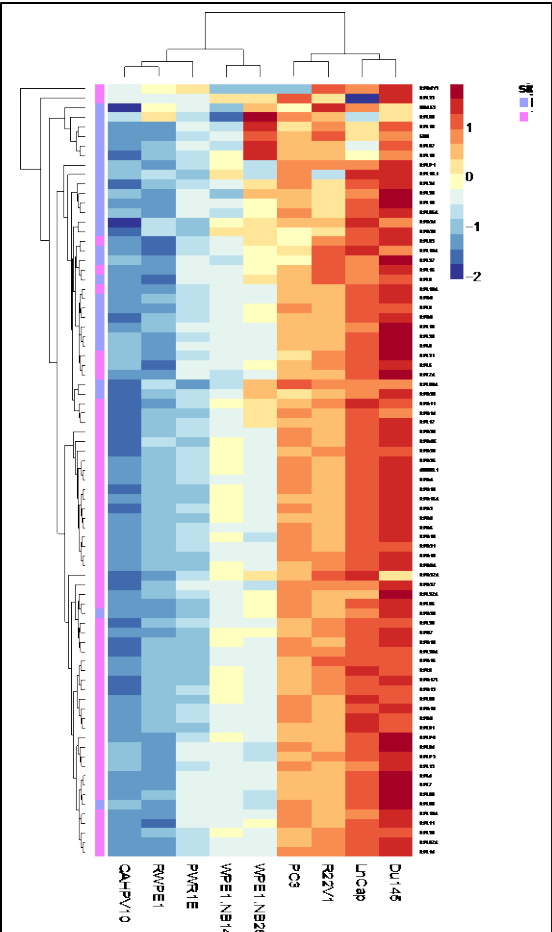


Figure 3. Analysis of ribosomal proteins in cancer cell lines. Data presented as normalized for the pooled reference control (average of 18 samples pooled together).

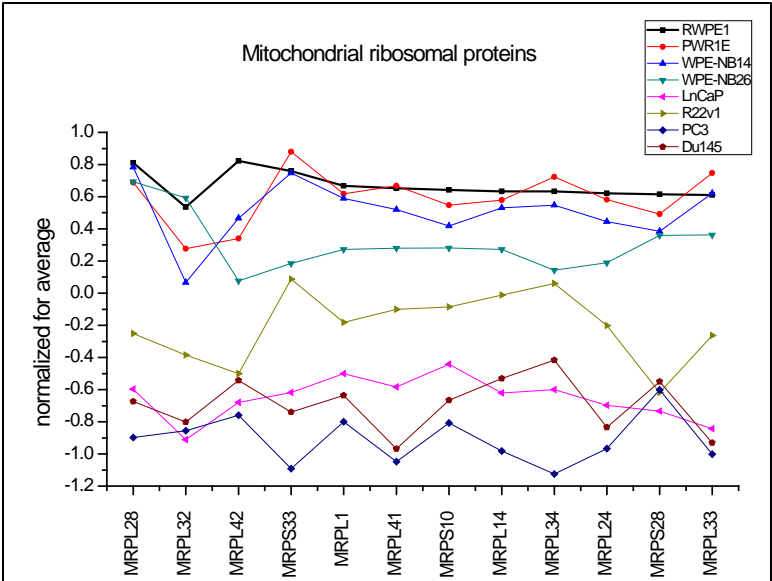


Figure 4. Example of mitochondrial ribosomal proteins down-regulation in cancer cell lines relative to RWPE1 and PWR1E normal cell lines (shown in black and red lines). Data presented as normalized for the pooled reference control (average of 18 samples pooled together)

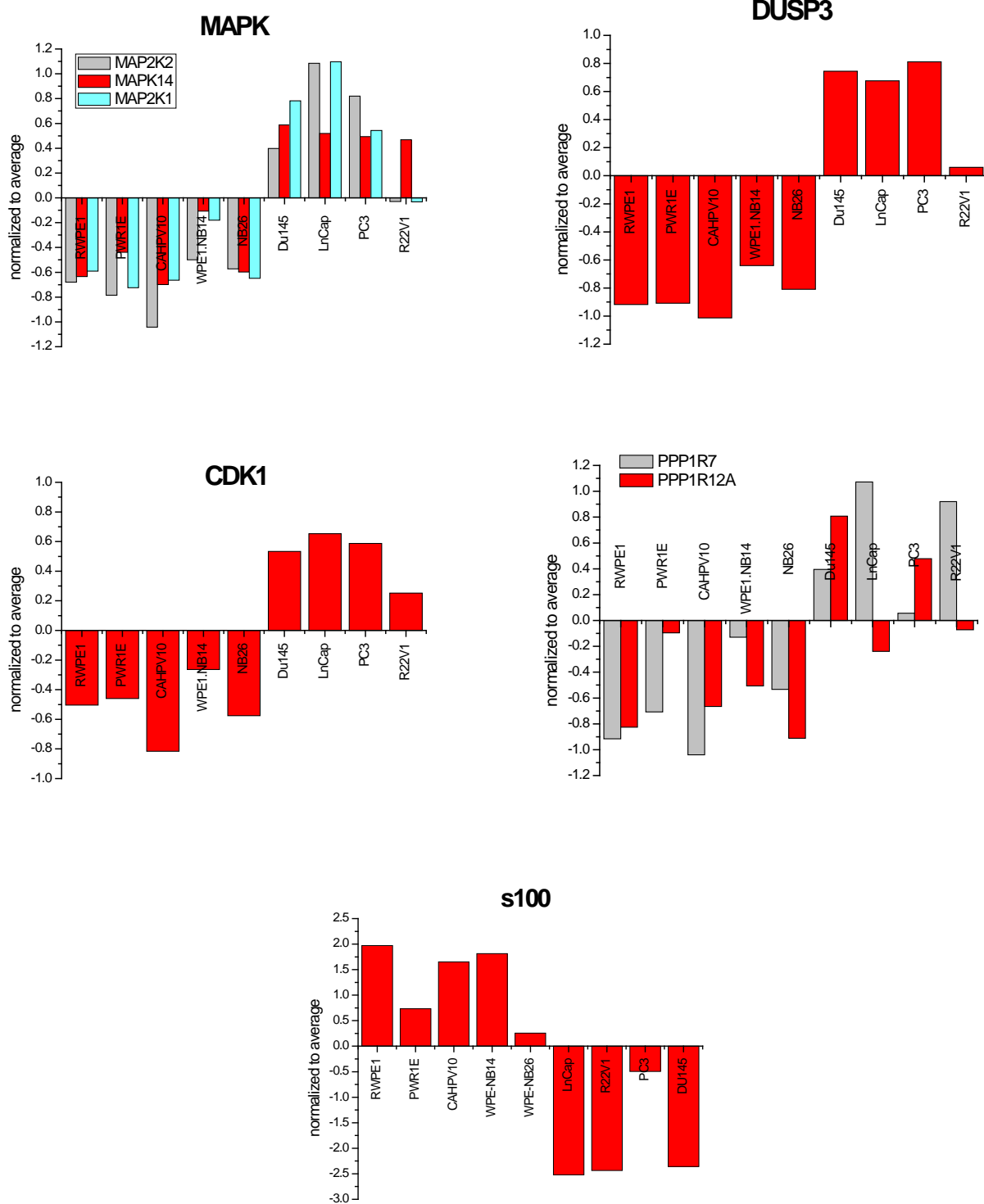
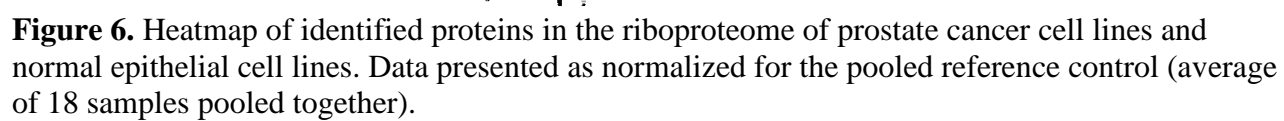


Figure 5. Heatmap of identified proteins in the riboproteome of prostate cancer cell lines and normal epithelial cell lines. Data presented as normalized for the pooled reference control (average of 18 samples pooled together).



On the other hand, S100A2, S100 calcium-binding protein A2, is significantly down-regulated in prostate cancer cell lines in comparison to RWPE1 cells (up to 4.5-fold decrease). It is also down-regulated in WPE1-NB14 and WPE1-NB-26 cells, which correlates with their degree of cancer progression (Fig. 5). S100A2 is a known tumor suppressor and is known to be down-regulated in a number of cancers, i.e., breast cancer and carcinomas. Several regulatory subunits of PPP1 are also upregulated in cancer cell lines (Fig. 5).

We further stratified the data based on androgen sensitivity of each cell line to analyze the changes that may dictate the response to androgen and be an important mechanism in the development of resistance to the therapy (Fig. 7), an Aim as outlined above in our proposal.

Our analysis identified a number of pro-oncogenic and tumor-suppressor proteins, which were not previously known to be associated with the riboproteome, one of them being MARCKS.

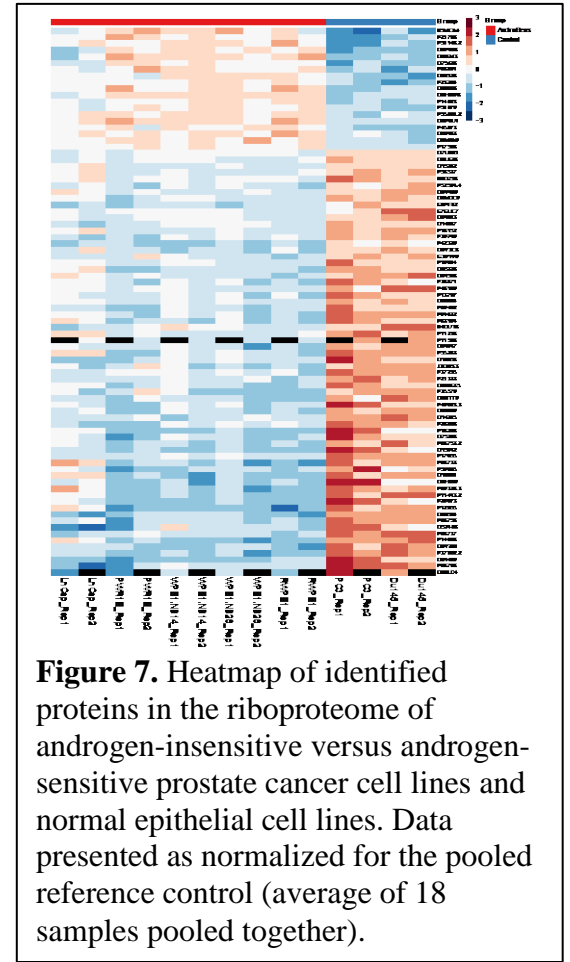
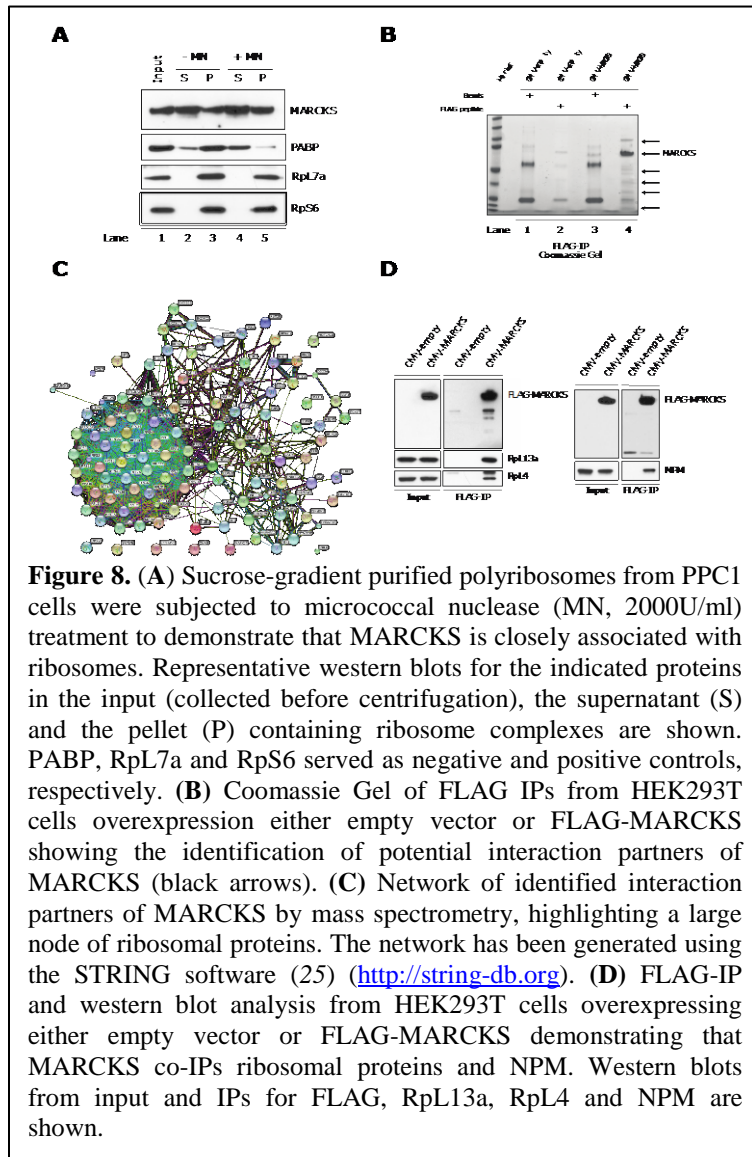


Figure 7. Heatmap of identified proteins in the riboproteome of androgen-insensitive versus androgen-sensitive prostate cancer cell lines and normal epithelial cell lines. Data presented as normalized for the pooled reference control (average of 18 samples pooled together).

Having identified MARCKS as a riboproteomic component we have worked to understand how this protein may function in cellular translation.

To demonstrate that MARCKS was directly associated with ribosomes we next employed a micrococcal nuclease (MN) assay previously described and optimized (Darnell et al., 2011). Treatment of sucrose-gradient purified polyribosomes with MN, followed by separation of ribosomes from released material by ultracentrifugation, revealed that a significant proportion of MARCKS reproducibly pelleted with ribosomes (Figure 8A, lanes 3 and 5). MARCKS was also observed to be present in the supernatant, even in the absence of MN (Figure 8A, lanes 2 and 4), which is in full agreement with the fact that this protein has additional known functions independent of ribosomal interaction. As expected, MN treatment readily released the RNA binding protein PABP from polyribosomes whereas MN did not affect the ribosomal proteins Rpl7a and Rps6 (Fig. 8A) (Darnell et al., 2011).

To further confirm the interaction of MARCKS

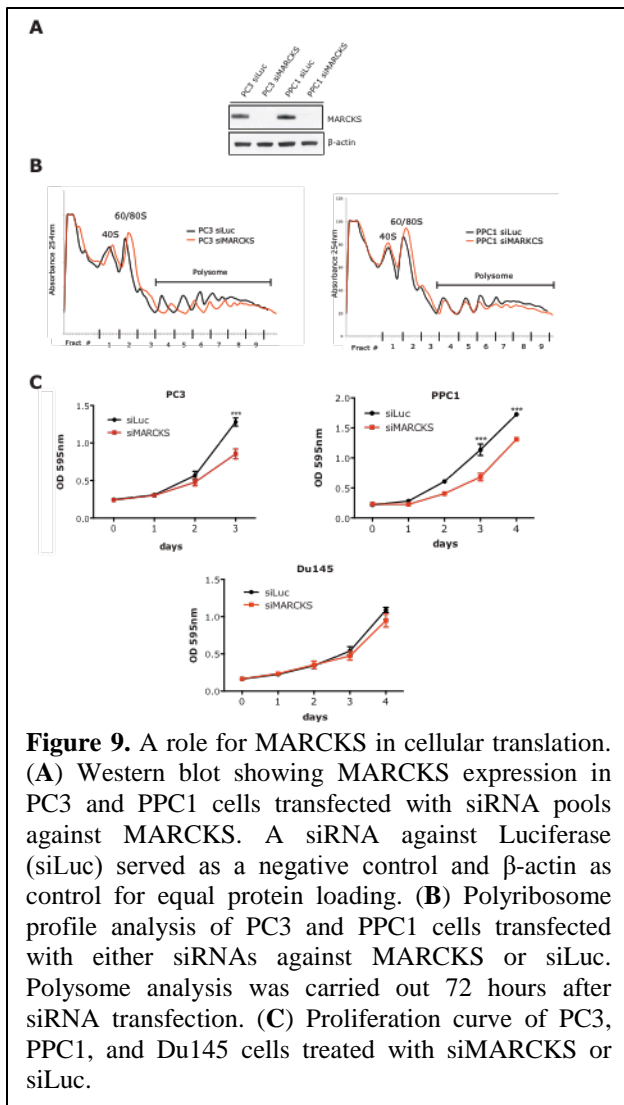


Figure 9. A role for MARCKS in cellular translation. (A) Western blot showing MARCKS expression in PC3 and PPC1 cells transfected with siRNA pools against MARCKS. A siRNA against Luciferase (siLuc) served as a negative control and β -actin as control for equal protein loading. (B) Polysome profile analysis of PC3 and PPC1 cells transfected with either siRNAs against MARCKS or siLuc. Polysome analysis was carried out 72 hours after siRNA transfection. (C) Proliferation curve of PC3, PPC1, and Du145 cells treated with siMARCKS or siLuc.

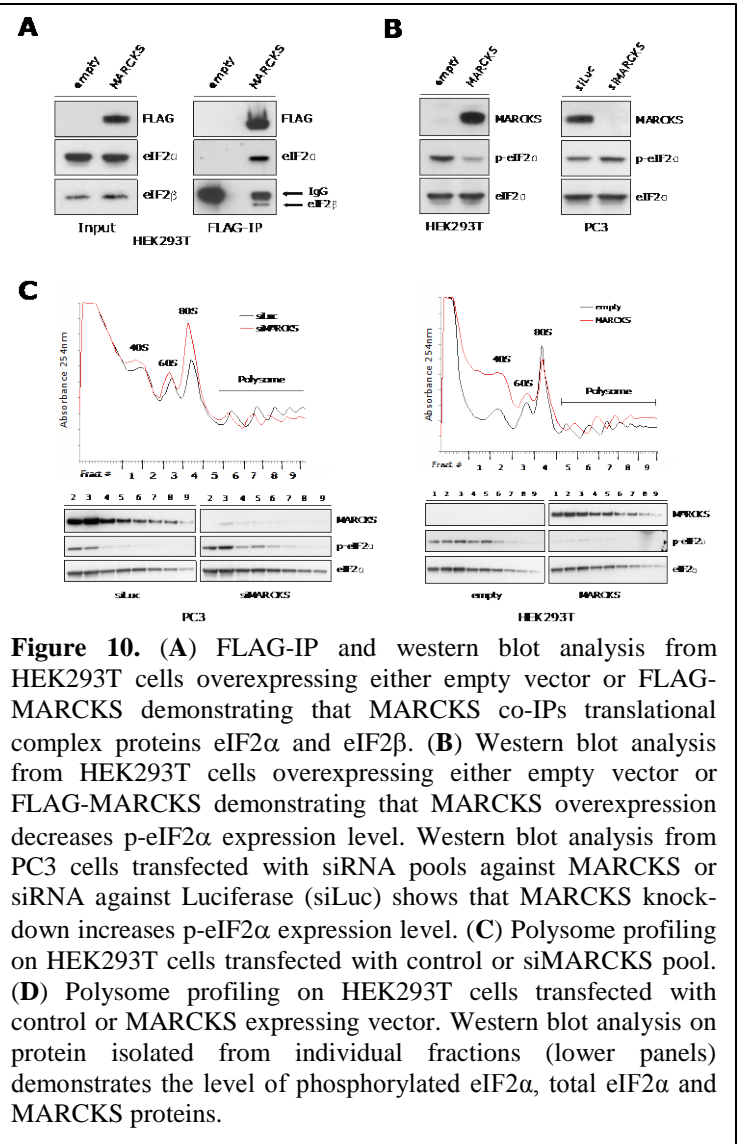
with ribosomes we over-expressed FLAG-tagged MARCKS in HEK293T cells to identify interaction partners by FLAG-immunoprecipitation (IP) followed by mass spectrometry (Fig. 8D). MARCKS and several interaction partners were efficiently eluted from the beads using a competitive FLAG peptide when compared to empty vector controls (Fig. 8D, lane 4). Strikingly, mass spectrometry analysis revealed a number of ribosomal proteins and ribosome-associated proteins as MARCKS interaction partners (Fig. 8C). Furthermore, western blot analysis confirmed that MARCKS co-immunoprecipitated with Rpl13a and Rpl4-containing complexes as well as with NPM, a protein known to be involved in ribosome biogenesis (Fig. 8D) (Grisendi et al., 2006; Maggi et al., 2008). These data further validate the association of MARCKS with the ribosome, and in turn support the hypothesis that MARCKS may contribute to the regulation of cellular translation.

In order to determine the role of MARCKS in tumorigenesis and translation, we used siRNA pools to deplete MARCKS from PC3 and PPC1 prostate cell lines (Fig. 9A). Strikingly, suppression of MARCKS resulted in a notable decrease in the amount of polyribosomes in both prostate cancer cell lines when compared to control transfected cells (Fig. 8B). In line with the decrease in translational output, we observed an inhibition of cell proliferation in both cell lines upon suppression of MARCKS (Fig. 9C). Notably, knockdown of MARCKS in Du145 cells, which contain little or no MARCKS at the polysome, did not result in evident growth suppression (Fig. 9C), confirming specificity of the siRNA reagent towards MARCKS.

These data prompted us to consider a role for MARCKS in the regulation of global translation, especially given its strong interaction with the numerous ribosomal proteins described above (Fig. 8C and 8D). Intriguingly, we identified eIF2 β , a component of the eIF2 translation initiation complex, as part of the MARCKS interactome. Thus, we chose to examine in greater detail the ability of MARCKS to interact and regulate the eIF2 translation initiation complex. To do this, MARCKS was overexpressed in HEK293T cells and immunoprecipitation experiments revealed the protein to interact with both eIF2 α and eIF2 β (Fig. 10A). The eIF2 α subunit is phosphorylated upon various stress stimuli, thereby inhibiting its ability to promote translation (Silvera et al., 2010). We thus hypothesized that MARCKS may favor the activity of the eIF2 initiation complex, and in this way contribute to translational regulation. Indeed, western blot analysis from MARCKS overexpressing HEK293T cells demonstrated a clear reduction in the phosphorylation of the eIF2 α subunit (Fig. 10B, left panel). Consistent with this finding, the knockdown of the MARCKS protein in PC3 cells shows an increase in the phosphorylation of eIF2 α (Fig. 10B). These data suggest that MARCKS may help protect cells from stress induced signaling, by directly interacting with the eIF2 translation initiation machinery and blocking inactivation of this complex. To confirm that the effects of MARCKS overexpression on eIF2 α phosphorylation actually takes place on the polysome, we next carried out polysome profiling on HEK293T cells transfected with control or MARCKS expressing vector (Fig. 10D). Overexpression of MARCKS was seen to promote polysome formation (in contrast to knockdown observations in Fig. 10C), with accumulation of ribosomal subunits and a concomitant decrease in 80S monosome (Fig. 10D, upper panel). On the other hand, MARCKS knockdown shows a reverse effect on polysome formation (Fig. 10C). Western blot analysis on protein isolated

from individual fractions demonstrated a clear reduction in the level of phosphorylated eIF2 α , while total eIF2 α protein levels remain constant when comparing control and MARCKS overexpressing cells, and the cells with knockdown MARCKS levels or control (Fig. 10C and D, lower panels).

Therefore, we identified a number of proteins previously not known to be associated with actively translating ribosomes (e.g. MARCKS, Integrin α 1 and ICAM1). Within this group, we validate MARCKS as a novel regulator of translation, and a potential biomarker. We show that MARCKS can specifically associate with polyribosomes and that it is required for efficient polyribosome formation and cancer cell proliferation. MARCKS impacts cellular translation through both direct binding to the ribosome, to translation initiation factors and to ribosome-associated proteins. Importantly, we find that through these interactions MARCKS regulates both global and specialized translation. The function of MARCKS in cancer cells, at least in part, may be to maintain active translation in the context of cellular stress, through its ability to interact with the eIF2 translation initiation complex. Interestingly, cellular stress and PKC can regulate MARCKS expression levels (Seitzer and Pandolfi, manuscript in preparation), and through its ability to regulate the phosphorylation of eIF2-alpha MARCKS functions to maintain translation under stressful conditions, highlighting its role as an important stabilizer of global translation and its proto-oncogenic potential. This is of particular relevance in oncogenic conditions, which are often accompanied by high levels of intracellular oxidative stress (Sosa et al., 2013).



What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

This is a final report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Deregulation of translational control marks a key event in prostate cancer development and it is well established that the malignancy of cells is strongly linked to and dependent on aberrant protein synthesis. Current knowledge clearly highlights deregulation of protein synthesis, in the development of prostate cancer, through aberrant activation of classical signaling pathways. It has been also hypothesized that aberrant composition of the translational apparatus itself (i.e. the composition of ribosomal and ribosome-associated proteins) can contribute to the transformation process. To date, however, the lack of an experimental platform to study the composition of the ribosome and its associated proteins in a high-throughput and systematic manner has impeded the validation of this hypothesis. Therefore, our research addresses this outstanding issue and provides a robust TMT-based mass spectrometry platform to systematically analyze the riboproteome of prostate cancer cells.

What was the impact on other disciplines?

These data can have important implications for the role of translation in cancer in general, and may be extrapolated for the benefit and understanding of general mechanisms of translational control in the progression of this disease.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We decided to perform a TMT-based mass spec approach (iTRAQ) instead of SILAC approach, as it allowed us to perform multiplexing of 10 samples with high sensitivity. The use of the reference polysome lysate allowed us to directly compare the riboproteome of all prostate cancer cells and correlate the relative abundance of riboproteomic components between datasets.

Actual or anticipated problems or delays and actions or plans to resolve them

Delay in the project progress has been encountered due to the change of the principal personnel and the change

in the methodology and planning of iTRAQ-based mass spectrometry approach.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

Nothing to Report

Publications, conference papers, and presentations

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Pier Paolo Pandolfi
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month	1.2

worked:	
Contribution to Project:	Dr. Pandolfi has supervised the work carried out and planned and analyzed experiments
Funding Support:	

Name:	John Clohessy
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.04
Contribution to Project:	Dr. Clohessy has planned and analyzed experimental data
Funding Support:	

Name:	Yulia Shulga
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	7
Contribution to Project:	Dr. Shulga has performed work in the area of preparation of polysome samples for the mass spectrometry
Funding Support:	Canadian Institutes of Health Research

What other organizations were involved as partners?

Broad Institute of MIT and Harvard